Mitigating Effects of Beta-Carotene on Hydroponic Growth and Productivity of *Amaranthus Hybridus* L. under Aluminium Toxicity Induced Stress

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Abstract

Aluminium (Al) toxicity is one of the major sources of environmental stress that limit plant growth and productivity in many acidic soils, especially in the tropics and sub-tropics. Al toxicity subsequently leads to food insecurity in developing countries, especially in sub-Saharan Africa. Though plants can synthesize their antioxidants; the need exists to investigate whether under Al toxicity-induced stress; beta-carotene (β-Carotene) supplementation could ameliorate the stress situation and enhance growth and productivity. A 3×10^{-2} mM aluminium chloride ($AlCl_3$) at pH 4.6 was used to stress plants. β-Carotene was extracted from carrots and high-performance liquid chromatography (HPLC) was used to determine its peak absorbance at 295 nm. β-Carotene's activity was determined using the thiobarbituric acid method. The effects of 50 and 200 µM concentrations of β-Carotene on the hydroponic growth of *Amaranthus hybridus*, subjected to Al stress, were evaluated in this study. Pre- and post- β-Carotene treatments were applied to *A. hybridus* seedlings before and after Al treatment for 72 h. Results showed that post- β-Carotene treatments significantly ameliorated plants from Al stress when compared to pre- β-Carotene treatments. Higher doses of β-Carotene significantly increased leaf number, plant height, length and number of inflorescence, fresh and dry weights of shoot, root and inflorescence but significantly decreased root length. The present study suggests that plants of *A. hybridus* were susceptible to Al toxicity-induced stress and post-β-Carotene supplementation could significantly ameliorate the stress situation and enhance growth and productivity. However, intrinsic antioxidants could be adequate for plants not subjected to stress.

Introduction

Aluminium (Al) toxicity is an important growth-limiting factor for plants in acid soils below pH 5.0 and is responsible for shortages in food production (Blue and Dantzman, 1977; Alak and Adams 1979; De Carvalho et al. 1980; Foy et al. 1992; Seguel et al. 2013; Nunes-Nesi et al. 2014; Soto-Cerda et al. 2015; Rahman and Upadhyaya 2021) and major stress agents that reduce crop productivity and increase food insecurity in the tropics and Sub-Saharan Africa (Kochian 1995; Hede et al. 2001; Rengel et al. 2015). Al is the third most abundant element making up over 8% of the earth's crust (Inostroza-Blancheteau et al. 2012; Bhalerao and Prabhu 2013; Rengel et al. 2015; Schmitt et al. 2016; Jaskowiak et al. 2019).

The general population is exposed to Al from its widespread use in water treatment, food additive, various Al-based pharmaceuticals, toothpaste, antiperspirants, pollutants from electrical power stations, industrial activities and automobile exhaust as well as from Al containers/packaging materials and cooking utensils (Harris et al. 1996; Ma et al. 2001; Pournourmohammadi et al. 2008). Al is solubilized into toxic forms such as hexaaquaaluminium ($[Al(H2O)6]^{3+}$); mononuclear species $[Al(OH)^{2+}]$ or $[Al(OH)^{2+}]$; gibbsite $[Al(OH)_3]$; aluminate $[Al(OH)^{4-}]$; $AlCl_3$ and are generally referred to as Al^{3+} (Kinraide 1990; Abreu et al. 2003; Silva 2012; Schmitt et al. 2016). Soil pH, chemical structure and composition of soluble Al compounds in soil solution as well as the solution's ionic strength have played key roles in Al toxicity (Siecinska and Nosalewicz 2017). Al toxicity affects plants directly and is transferred to humans

and animals that consume these plants via the food chain (Fatur et al. 2002; Savvas et al. 2010; Rahman and Upadhyaya 2021).

Some of the most noticeable phytotoxic symptoms of Al toxicity include inhibition of root growth (Silva 2012; Kopittke et al. 2015; Rosmaninho et al. 2019), leaf-chlorosis, and stunted plant growth (Gupta et al. 2013; Vasconcelos et al. 2020). The roots exhibit greater signs of cellular damage than the other parts of the plant (Wagatsuma et al. 1987; Rincón and Gonzales 1992; Udengwu and Egedigwe 2014; Kochian et al. 2015). Under Al stress in a nutrient solution, Al-sensitive genotypes were characterized by chlorosis (Udengwu and Egedigwe 2014), decreased Fe concentration in tops, decreased/inhibited Ca and Mg uptake in both shoots and roots (Horst et al. 2010; Bose et al. 2011), a tendency towards the accumulation of P, Al, and Fe in roots and reduced Mn in tops (Foy and Fleming 1982; Gupta et al. 2013). Studies have shown that Al adversely affected several physiological activities, producing severe physiological stress which increased peroxidase activity (Peters et al. 1989; Kochian et al. 2015; Rengel et al. 2015; Rouphael et al. 2016). Al toxicity induces oxidative stress that elicits the production of reactive oxygen species (ROS) (Darkó et al. 2004; Jones et al. 2006; He et al. 2014; Singh et al. 2017) that may damage cellular components if surrounding antioxidant enzymes are suppressed (Darkó et al. 2004; Sharma and Dubey 2007; Bera et al. 2019; Shahnawaz and Sanadhya 2019; Devi et al. 2020). Cytological studies show that Al toxicity results in cell cycle disturbances and a decrease in Mitotic Index (MI) value (Wierzbicka 1988, 1989; Samardakiewicz and Woźny 2005; Nezames et al. 2012) as well as induces C-mitosis, micronuclei, chromosome stickiness, budding nuclei, laggards and chromosome bridges (Aslanturk and Celik, 2005; Zhang et al. 2014). Given the menace of Al toxicity, especially in the sub-Saharan region, current research is now geared towards proffering long-term solutions to mitigate the devastating effects of Al toxicity on crop yield and productivity.

It is pertinent to point out that studies have attributed low pH to be the major cause of stress and the ignition to the production of ROS (Samac and Tesfaye 2003; Inostroza-Blancheteau et al. 2012; Udengwu and Egedigwe 2014; Yang et al. 2015; Rahman and Upadhyaya 2021). Previous studies concerning Al toxicity have focused only on Al being the major cause of stress limiting crop productivity but in reality, low pH sets the stage for Al to become toxic. Al toxicity not only depends on its total concentration but also its chemical forms that wholly depend on low pH (Kochian 1995). Hence, there is a significant correlation between high concentrations of $Al3+$ in the soil and low pH (Rout et al. 2001; Bojórquez-Quintal et al. 2017; Palani et al. 2018).

There is growing evidence from *in vitro* and *in vivo* laboratory animal studies that β-Carotene can protect phagocytic cells from auto-oxidative damage, enhance T and B lymphocyte proliferating responses, stimulate effector T-cell functions, and enhance macrophage, cytotoxic T- cell and natural killer cell tumoricidal capacities, as well as increase the production of certain interleukins (Bendich 1989; Mueller and Boehm 2011; Esrefoglu et al. 2016). Many of these effects have also been seen with carotenoids lacking provitamin A activity but having the antioxidant and singlet oxygen quenching capacities of β-Carotene, however, reports have it that β-Carotene could be converted into vitamin A, which is essential for normal growth and development (Lemmens et al. 2010; Giuliano et al. 2017)). Since vitamin A is a

relatively poor antioxidant and cannot quench singlet oxygen, β-Carotene may have more importance as a nutrient than simply serving as a precursor of vitamin A (Bendich 1989). Carotenoids, including β-Carotene, are very efficient at quenching singlet oxygen (Di Mascio et al. 1989; Baltschun et al. 1997).

Stahl and Sies (2003) have shown that β-Carotene undergo different configurations (either cis or trans isomers) because of their double bonds. Growing observations have also showed that most carotenoids isomerize to their cis-configuration during thermal procession and extraction (Lemmens et al. 2010), storage conditions due to light acids and oxygen (Rao and Rao 2007), thus leading to loss of colour and reduction in biological activities. However, the all-trans isomer may exist predominantly in nature.

Though evidence for carotenoids roles' in animals and humans are ubiquitous, studies about the supplementation of plants with β-Carotene are scarce. However, lycopene had ameliorating effects on chromosome aberrations in *Allium cepa* (Aslanturk and Celik 2005); while a higher concentration of lycopene alleviated and bolstered plants of *A. hybridus* from the devastating effects of Al-induced stress (Udengwu and Egedigwe 2014). It is well known that animals cannot synthesize carotenoids because they lack chromoplasts and thus depend on plants for the nutritious and protective values of carotenoids. However, Moran and Jarvik (2010) showed that some aphids manufacture toluene *de novo*.

A. hybridus, being a staple vegetable eaten in the tropics, is sensitive to low concentrations of Al and such low concentrations subjected the same plant to stress and reduced growth and productivity (Udengwu and Egedigwe 2014). In another study, Osaki et al. (1997) classified *A. hybridus* and some other tropical plants under the Al-sensitive group. Despite the intrinsic antioxidants inherent in plants, some plants, including staple crops, may not be capable of synthesizing high levels of antioxidants during stress situations and thus cause devastating damages to plant growth and yield. Excessive reduction in crop yield and productivity have consequently led to food insecurity in sub-Saharan regions. The current study, therefore, explores the role of supplementary β-Carotene in mitigating plants of *A. hybridus* exposed to Al toxicity via pre- and post- β-Carotene applications.

Materials And Methods

Soil Analysis

Topsoil for raising nursery plants was collected from the Botanic Garden, University of Nigeria Nsukka. The soil sample was air-dried and analysed in the Soil Science Analytical Laboratory in the Department of Soil Science, University of Nigeria Nsukka. The soil was analysed using the standard method of the Association of Official Analytical Chemists (2005).

β-Carotene extraction and purification

Extraction and purification of β-Carotene were carried out following the methods of Udengwu and Egedigwe (2014). Fresh and matured carrots weighing 15kg were purchased from Nsukka local market. β-

Carotene crystals were got following the methods of Yaping et al. (2002). Extracted β-Carotene was protected from light and stored at 2^{o}C to avoid transformation to inactive isomers.

Determination of antioxidant activity

Adapting the methods of Udengwu and Egedigwe (2014), thiobarbituric acid (TBA) value was used to determine the antioxidant activity of β-Carotene. All readings were taken thrice and antioxidant activities were calculated as follows using the method of Hanachi and Sh (2009).

% Antioxidant Activity = $\frac{ABcontrol - ABsample}{ABcontrol} \times 100$

Where AB_{sample} = Absorbance of sample and $AB_{control}$ = Absorbance of control

High-performance liquid chromatography analysis (HPLC)

The percentage (%) purity of the extracted β-Carotene was determined using the methods of Udengwu and Egedigwe (2014). HPLC was done in the Department of Pure and Industrial Chemistry, University of Nigeria Nsukka, while UV spectrometry was determined using a UV-visible spectrophotometer. Readings of β-Carotene standard in ethanol were taken to confirm the peak absorbance of extracted β-Carotene.

Stock preparations

Fresh 1 M stock solutions of β-Carotene, $AlCl_{3,}$ and full Hoagland's nutrient were prepared daily using the methods of Udengwu and Egedigwe (2014). They were stored at 4^{o}C in the refrigerator before use.

β-Carotene stock solution: One gram of β-Carotene was mixed in 10 mL of ethanol before the addition of 990 mL of distilled water. A 1% alcohol dilution of β-Carotene was used in this study following the protocol of Fiskesjo (1981); who showed that 1% of alcohol dilutions of lipophilic solutes were not toxic to *Allium* roots.

$AlCl_3$ stock solution (1 Molar)

This was prepared by dissolving 133.5g of $AlCl_3$ in little distilled water and the final volume made up to 1000 mL. The pH of the solution was buffered to 4.6.

Al treatment concentration

Al treatment concentration of 3×10^{-2} mM was achieved through serial dilution and pH 4.6 was through adjustments with H_2SO_4.

Hoagland's nutrient solution

This was prepared using the formulation of Hoagland and Arnon (1950 revised).

Determination of actual Al concentration in solution

Actual Al in solution was determined using Shull (1960) modified Aluminon method for aluminium determination.

Growing Amaranthus hybridus

Viable *Amaranthus* seeds were obtained from the *Amaranthus* germplasm maintained in the Botanic Garden, University of Nigeria, Nsukka. Seeds maintained in the germplasm were acquired from National Horticultural Research Institute (NIHORT), Ibadan Nigeria. The exact methods of Udengwu and Egedigwe (2014) were used to grow *Amaranthus* plants. Plants were arranged using a randomized complete block design (RCBD) in the screen house of the Botanic garden. Five plants per treatment, replicated three times, were used to monitor the growth of plants. Plants were allowed to stabilize in full strength Hogland's nutrient solution for 10 d before the 8 treatments were applied (Table 1). All plants received treatments for 21 days. Using a non-continuous flow system, all treatments were renewed daily to ensure adequate nutrient supply and uniformity of treatment. When treatment application elapsed, the experiment was terminated. Fresh and dry weights of shoots, roots and inflorescences, as well as other growth parameters such as the numbers of leaves and inflorescences, plant height, root length, length of inflorescence, were evaluated and recorded.

Table 1
Details of the 8 Treatments given to experimental plants

S/N	Type of Treatment	Abbreviation	Symbol	Details
1.	β-carotene (50μM)	bc_1	T1	*Amaranthus* plants (AP) were grown in Hoagland's nutrient solution (HNS) and 50μM bc_1 for 21 d
2.	β-carotene (200μM)	bc_2	T2	AP was grown in HNS and 200μM bc_2 for 21 d
3.	Pre-β-carotene (50μM)	(bc_1-Al)	T3	AP was grown in HNS and 50μM bc_1 for 72 h before transfer into HNS and 3 x 10^{-2} mM Al for 18 d.
4.	Pre-β-carotene (200μM)	(bc_2-Al)	T4	AP was grown in HNS and 200μM bc_2 for 72 h before transfer into HNS and 3 x 10^{-2} mM Al for 18 d.
5.	Post-β-carotene (50μM)	(Al-bc_1)	T5	AP was grown in HNS and 3 x 10^{-2} mM Al for 18 d before transfer into 50μM bc_1 for 72 h.
6.	Post-β-carotene (200μM)	(Al-bc_2)	T6	AP was grown in HNS and 3 x 10^{-2} mM Al for 18 d before transfer into 200μM bc_2 for 72 h.
7.	Aluminium (3 x 10^{-2} mM)	Al	T7	AP grown into HNS and 3 x 10^{-2} mM for 21 d
8.	Control	Ctrl	C	AP grown into HNS for 21 d

Statistical analysis

Data collected from growth studies were analysed with one-way analysis of variance (ANOVA). The Least Significant Difference (LSD) was used to separate means at $P \leq 0.05$ level of significance. SPSS v23, Microsoft Excel 2016 and Gen-Stat packages were used for computation, data analysis and graphics.

Results

Soil analysis

The result of soil analysis was the same as the results of Udengwu and Egedigwe (2014). Results showed no presence of Al (Table 2).

Table 2
Physical and chemical composition of the topsoil in the Botanic Garden, University of Nigeria, Nsukka, used for seed germination and seedling production (Udengwu and Egedigwe 2014)

Parameters	Values
pH (H_2O)	6.200
pH (KCl)	5.200
Fine soil (%)	29.000
Silt (%)	5.000
Clay (%)	27.000
Coarse soil (%)	39.000
Organic matter (%)	5.779
Organic Carbon (%)	3.352
Total Nitrogen (%)	0.168
Available P (ppm)	37.370
Exchangeable cations (mg/100g)	31.200
Calcium (mg/100g)	8.800
Magnesium (mg/100g)	15.200
Sodium (mg/100g)	0.501
Potassium (mg/100g)	0.131
Hydrogen ion (mg/100g)	3.400

Antioxidant activity

The antioxidant activity of β-Carotene, 72%, differed significantly from ethanol blank, which exhibited a lower percentage activity. Rhee (1978) explained that the TBA test was a colorimetric technique used in measuring the absorbance of a red chromogen formed between TBA and malondialdehyde (MDA). Peak absorbance of extracted β-Carotene at 295nm was 0.181.

Hydrogen ion concentration

Using the formula of Stephenson (2010), the H^+ concentration of extracted β-Carotene at pH 5.8 was determined by taking 10 to the power of the negative pH i.e., $10^{-5.8}$ which is equivalent to 1.5×10^{-6} mol L^{-1} while the H^+ concentration of 3×10^{-2} mM of Al at pH 4.6 was calculated to be 2.5×10^{-5} mol L^{-1}.

Determination of Actual Al concentration in solution

Following the Aluminon protocol, the actual Al concentration in solution used for the study was 1.85 mg/L.

Vegetative and reproductive data.

Before treatment applications, *Amaranthus* plants stabilized and were acclimatized in full strength Hoagland's nutrient solution (Fig. 1). The first noticeable symptoms observed three days after Al treatment applications, which was the same as the results of Udengwu and Egedigwe (2014), were prominent inhibition of root growth (Fig. 2), yellowing and wilting of leaves and overall stunting plant growth (Fig. 3).

Number of leaves (NOL) At the end of 21 days, Al-bc_2 (T6) significantly yielded a higher number of leaves in comparison with other treatments except control (Ctrl). Only T6, a post-β-Carotene treatment, significantly yielded more leaves than that observed in pre-β-Carotene treatments, bc_1-Al (T3) and bc_2-Al (T4). Al treatment (Al) (T7) significantly reduced the number of leaves by 44.1% in comparison with the control (Fig. 4).

Plant height (PLH) Increasing concentrations of post-β-Carotene treatments, Al-bc_1 (T5) and T6, significantly increased plant height compared to increasing concentrations of pre-β-Carotene treatments (T3 and T4). Figure 5 shows that in T7, there was a significant reduction (36.5%) in PLH when compared with control plants (Fig. 6).

Length of inflorescence (LOI) Fig. 7 shows that the least LOI was recorded with T3. Lengths of inflorescences from all treatments differed significantly from T6. Al (T7) significantly reduced the length of inflorescence by 37%.

Number of inflorescences (NOI) As shown in Fig. 8, T6 yielded the highest number of inflorescence and differed significantly from other treatments. T7 significantly reduced the number of inflorescences. Treatments, bc_2 (T2) and T3 significantly reduced the number of inflorescences by 88.6% and 95.2% respectively. Post-β-Carotene treatments (T5 and T6) yielded a higher number of inflorescences when compared to pre-β-Carotene treatments (T3 and T4).

Fresh weight of inflorescence (FWI) Responses of plants to all treatments in Fig. 9 significantly reduced FWI in comparison with control. There were no significant differences in FWI between pre-β-Carotene and post-β-Carotene treatments, except T6. Having a similar trend with the results got in LOI and NOI, T2 and T3 significantly reduced FWI by 86.5% and 97% respectively.

Dry weight of inflorescence (DWI) Responses of plants to treatments in Fig. 10 followed the exact trend with plants responses to treatments in FWI. The reduction in DWI was not significant in comparison with that of control plants. Responses of plants to T6 and T7 did not differ significantly from each other but differed significantly from other treatments, including control.

Fresh weight of shoot (FWS) Decreasing pre-β-Carotene concentrations increased FWS, however, increasing post-β-Carotene concentrations were directly proportional to a significant gain in FWS. All treatments significantly reduced FWS except T6. Responses of plants to T6 significantly differed from that of T1, T2, T3, T4, T5 and T7 (Fig. 11).

Dry weight of shoot (DWS) Fig. 12 shows that decreasing pre-β-Carotene concentrations increased DWS while increasing post-β-Carotene concentrations significantly increased DWS. Only T6 differed significantly from other treatments except for the control. Though not significant, responses of plants to T1 and T2 yielded higher DWS compared to T3, T4, T5 and T7. The least DWS was produced with T4.

Root length (ROL) Root length of control plants differed significantly from the rest of the treatments. The response of plants to bc_1 (T1) significantly reduced ROL and differs significantly from plants responses to T2, T4, T5 T6, T7 (Fig. 13) and control (Fig. 14). The least ROL was recorded with T6. Decreasing β-Carotene concentrations across treatments were directly proportional to longer ROL. Though there were no significant differences, pre-β-Carotene treatments prolonged root lengths more than post-β-Carotene treatments.

Fresh weight of root (FWR) Increasing concentrations of pre-β-Carotene treatments did not significantly increase FWR except in post-β-Carotene gain in concentration (Fig. 15). There was no significant reduction by T7 in comparison with control. Responses of plants to T1 and T6 increased FWR by 20% and 68.2% respectively.

Dry weight of root (DWR) The responses of plants to T3 and T5 recorded the highest and least DWR respectively compared to Control (Fig. 16). T7, T6, T1 and T3 treatments increased DWR by 13.5%, 18.9%, 41.8% and 131.3% respectively. Increasing β-Carotene concentrations increased DWR except in post-β-Carotene increment.

Discussion

The results of soil analysis used in this study are the same as the results of Udengwu and Egedigwe (2014). It follows that Al was completely absent in the soil used and that aluminium chloride ($AlCl_3$) is the only source of Al used in this study. In comparison with the study of Udengwu and Egedigwe (2014), the findings of this study still showed that Al, at a low concentration of 3×10^{-2} mM and low pH of 4.6, significantly reduced both vegetative and reproductive growths of *A. hybridus* (Inine oma) except in FWR, DWR and DWI. This conforms to the findings of other studies that Al toxicity may not affect plants except under low pH conditions (Moore 1974; Dickson 1978). In essence, Udengwu and Egedigwe (2014) grouped *A. hybridus* as an Al-sensitive plant in the tropics. Tamás et al. (2006) reported that *Hordeum vulgare* (barley) roots represented only 20% of the control when plants were subjected to 10mM Al for 12 h. Plants were grown in Hoagland's nutrient solution to eliminate uncertainties of roots absorbing unknown amounts of different nutrient elements and other toxic metals if grown in soil. There is also an added advantage of the ease in controlling the pH. Using transparent bottles allowed for full monitoring

of roots during growth and unwanted cutting of root tips if grown in soil. Despite these advantages, it is important to mention that the growth of *Amaranthus* plants in nutrient solution will not be as robust as when grown in soil because *A. hybridus* is not an aquatic plant. It is quite interesting to point out the similar trend plants responded to the respective treatments via the growth parameters except for the situations in ROL and DWR. This shows that responses of experimental plants to independent β-Carotene treatments (T1 and T2) and pre-β-Carotene treatments (T3 and T4) were, perhaps, redundant until Al (T7) was applied (T6). The response of T7 in comparison with T6 significantly reduced growth for NOL, PLH, LOI, NOI, FWS, FWR, and DWS but was non-significant for DWR and DWI. Udengwu and Egedigwe (2014) attributed the decrease in growth to prior treatment of *A. hybridus* plants to Al for 21 d at 4.6 pH and higher H^+ concentration of 2.5×10^{-5} mol L^{-1} before transfer to lycopene solutions for only 72 h, at a pH of 5.8 and lower H^+ concentration of 1.5×10^{-6} mol L^{-1}. Interestingly, Al-stressed plants grown in T5 and T6 recovered significantly for NOL, PLH, LOI, NOI, FWS, FWR and DWS, but non-significantly for FWI, DWR and DWI. From the findings of Udengwu and Egedigwe (2014), lycopene did not ideally recover plants from Al stress as much as β-Carotene ameliorated Al-stressed plants in this study. It could then be deduced that β-Carotene possesses a higher antioxidant power in combating Al-related stress, though both lycopene and β-Carotene contain the same number of conjugated double bonds, as well as β-Carotene, being a derivative of lycopene. Several studies have attributed β-Carotene's anti-oxidative activity to its high number of conjugated double bonds (Woodall et al. 1997; Muller and Boehm, 2011). Miller et al. (1996) reported that the properties underlying the activities of carotenoids towards free radicals and their scavenging effects related particularly to their abilities to donate electrons or hydrogen atoms and their relative propensities to undergo oxidation. Further studies are needed to explore whether the functional groups on the β-ionone ring of β-Carotene are responsible for the extra antioxidant activity of the carotenoid compared to lycopene.

Increased concentration of independent β-Carotene treatments (T1 and T2), though not significant, reduced growth for all growth parameters except for ROL, where a significant reduction in growth was recorded with increased β-Carotene dosage. On the other hand, with the introduction of Al stress (T3 to T6), β-Carotene's ameliorative effect was non-significant in plant growth (for all parameters except ROL and DWR) when concentrations were increased. It could be possible that the activity of β-Carotene becomes functional upon inducement of Al stress and that the Al-stressed plants absorbed the supplemented β-Carotene because intrinsic and stress-induced antioxidant enzymes were not enough for the stressed plants to recover from the stress condition caused by Al toxicity (Udengwu and Egedigwe 2014). Thus, the generation of antioxidant enzymes and antioxidant response elements (ARE) has been strongly linked to stressed environments (Boscolo et al. 2003; Siminovicova, 2004; Sharma and Dubey 2007; Aftab et al. 2010). The significant increase in plant growth of post-β-Carotene treatments (T5 and T6), (in almost all parameters except ROL and DWR), was more than that observed in pre-β-Carotene treatments (T3 and T4). The major reason for this has been attributed to stressed plants not needing β-Carotene supplementation when not stressed (T1 and T2) until the introduction of Al stress (T3 and T4) (Udengwu and Egedigwe 2014). They explained that *A. hybridus* that are not under stress may not need supplemented antioxidants because they are adequately protected by their intrinsic antioxidants and that

these antioxidants mitigate stress conditions and not serving as growth stimulants. From the results obtained, it is suggestive that an increased dose of β-Carotene concentration (T2) given to plants for 72 h after Al stress yielded a significant ameliorative effect for all cases except ROL and DWR. This suggests that *A. hybridus* plants significantly absorbed β-Carotene when under Al stress and not before Al stress. Perhaps, β-Carotene may have yielded a better ameliorative effect when the dose was further increased (Udengwu and Egedigwe 2014). The difference in plant growth of T1 in comparison with T2 was significantly higher for only ROL, but was insignificant for other growth parameters. Having in mind that T1 and T2 each had a pH of 5.8 and H^+ concentration of 1.5×10^{-6} mol L^{-1}, the reason for the lower insignificant growth decrease of T2 over T1 could be that T1 was optimum in ameliorating stress (caused by H^+) better than T2 where β-Carotene activity could have been redundant. This could buttress the assumption that plants need not be supplemented with β-Carotene except under stress conditions. The effect of Al in plants receiving T3 and T4 showed there was a significant growth decrease in the scenarios for PLH, LOI, NOI, FWI and DWI, however, β-Carotene significantly improved Al stress in the case of DWR. The possible reason for this is that transferring plants to Al (T7) (treated for 18 d) from β-Carotene solution (treated for only 72 h) subjected plants in T3 and T4 (pre-β-Carotene treatments) to greater stress than plants that were transferred from β-Carotene solution to Al in T5 and T6 (post-β-Carotene treatments) (Udengwu and Egedigwe 2014). They explained further that a synergistic effect of both Al at lower pH of 4.6 and H^+ concentration were the real culprits to decreased plant growth.

The responses of *A. hybridus* plants to T6 regarding FWR showed a significant increase in plant growth while for NOI, an insignificant growth in plants was recorded. More so, β-Carotene significantly ameliorated Al stress for these parameters (NOI and FWR). This shows that the intrinsic antioxidants within plants of *A. hybridus* could not protect them against stress generated from Al toxicity; rather, plants needed a higher dose of β-Carotene to combat the stress condition. This suggests that under Al stress, the ARE in these plants was increased with higher β-Carotene concentration. Zhang et al. (2010) found that enzyme activities of amylases and esterases, which decreased the levels of MDA and hydrogen peroxide (H_2O_2), were increased in wheat plants under stress when pre-treated with H_2S. In the situation for DWR, only plants that responded to T3 showed a significant increase in plant growth when compared to control and Al treatments. The reason for this is not known, however, there is a possibility that β-Carotene could play important roles in decreasing the uptake of Al ions because β-Carotene readily binds to the free hydroxyl groups of Al, forming β-Carotene -Al complexes (radical adducts and cations), thus reducing free Al that would have been available for root absorption. It is, therefore, possible that β-Carotene could have played vital roles in boosting roots mechanisms of either excluding or chelating Al ions. Nevertheless, Al may exhibit an unpredictable interaction with mineral nutrients and β-Carotene, thus undergoing varying actions in connection with an increase or decrease in concentration when in solution. Al could also affect the secretion or suppression of different hormones that are responsible for different functions in plant growth. The controversy will be whether β-Carotene interacts with Al in a fashion that up-regulates or down-regulates the production of specific hormones at specific time frames towards the growth of plants.

Visible symptoms such as leaf stunting, chlorosis and death were characteristic in plants after 9 days of Al treatment. Al could have possibly interfered with essential mineral nutrient uptake in the plants. Some studies have found that Al interference leads to deficiencies of mineral nutrients that consequently manifest symptoms of impaired growth (Delhaize and Ryan 1995; Chang et al. 1999). For all the vegetative and reproductive parameters studied, Al treatment significantly impaired plant growth apart from the FWR, DWR and DWI. It may suggest that plants of *A. hybridus* could have absorbed Al within their leaves, shoots, as well as seeds and there could be a possible transfer of Al, along with the food chain, to its consumers since *A. hybridus* is a staple vegetable source in Sub-Saharan Africa. Though the concentration of Al in different parts of the plant did not form part of this study, studies have shown an increase in the accumulation of Al and other heavy metals in their shoots and upperparts, inferring that such plants are hyper-metal accumulators without injury to the plants (Kukachka and Miller 1980; Watanabe and Osaki, 2002; Li et al. 2010). Though literal information on the mechanism, cellular localization and chemical form of Al, are meagre; (Jansen et al. (2002) gave reasons for such hyper-accumulative behaviour to either low/non-accumulation of Al in their shoots or an epiphytic habit. Other reasons were differences in soil pH, ectomycorrhizal association, possible acquisition of new Al-responsive mechanisms, and inherited ancestral traits. The present study revealed that the roots were the primary targets of Al toxicity as they were stubby, brittle, brownish and stunted. Similar results have been recorded by other studies (Zhang and Rengel 1999; Mossor-Pietraszewska 2001; Doncheva et al. 2005). Results show that Al stress significantly reduced ROL and that β-Carotene could not ameliorate the stress condition. This suggests that the apical roots gave rise to an extensive production of lateral roots as a response signal to Al stress. Llugany et al. (2003) showed that plants of *Silene armeria* responded rapidly by producing many lateral roots following Cu stress to the root tip meristem. They suggested such responses may prevent the extension of the major root into soil patches with high ion toxicity and favour the extension of lateral roots into the less toxic topsoil. Thus, the production of extensive lateral roots may be responsible for the insignificant increase in plant growth for DWR. Only the response of plants to T3 (pre-β-Carotene treatment) showed an insignificant ameliorative effect in combating Al stress. A probable reason could be that β-Carotene boosted roots' exclusion mechanism or exudation of chelating agents in preventing the accumulation of phytotoxic Al in the apoplast and symplast (Gill and Tuteja 2010).

β-Carotene and other carotenoids predominantly occur in their all-trans configuration, however, convert to their cis-isomers during its processing or perhaps when *in-vivo*. It is not known in this study on which isomer was bioavailable to *A. hybridus* plants, however, studies have shown that the cis-isomers, though susceptible to oxidation and may present lower bioactivity, are more bio-available to humans and animals after processing (Tyssandier et al. 2003; Khoo et al. 2011; Udengwu and Egedigwe 2014; Strati and Oreopoulou 2016). The situation may be different in plants but it is hypothesized in their study that the cis-isomer of lycopene (either *in-vitro* or *in-vivo*) ameliorated plants of *A. hybridus* from Al stress (Udengwu and Egedigwe 2014). This now raises the question whether the cis-isomer is transformed back to its all-trans configuration when *in-vivo*.

This study has shown that plants of *A. hybridus* responded positively to supplemented β-Carotene. Previous studies have concentrated on the roles of β-Carotene in non-photosynthetic organisms (animal models and humans) because neither animals nor humans can synthesize their antioxidants. Studies concerning the supplementation of *in vivo* β-Carotene in plants are meagre; however, studies on β-Carotene's role in activating and boosting the antioxidant activities of several plants have been reported. Plants' intrinsic antioxidants could be boosted by the application of certain compounds *ex vivo* or through the induction of stressful conditions. Tian et al. (2012) showed that spermicide alleviated the oxidative damage in cucumber seedlings subjected to high temperatures by enhancing the activities of antioxidant enzymes.

Conclusion

In conclusion, the present study has shown that under Al stress, plants of *A. hybridus*, 'Inine oma' suffer deteriorating effects if not protected or mitigated with increased concentration of β-Carotene. An interesting aspect of this work was that plants' fight against Al stress was bolstered by supplemented β-Carotene. Higher doses of post-β-Carotene treatment ameliorated Al stress more than other treatments. This is to say that β-Carotene is not only a precursor of vitamin A but may possess strong anti-oxidative properties against Al toxicity in *A. hybridus*. Independent β-Carotene treatments without Al stress that did not significantly affect growth suggested that plants may not require extra antioxidant supplementation unless subjected to Al stress as obtained in pre-and-post-β-Carotene treatments. From the results, it was evident that plants of *A. hybridus* were not tolerant to Al stress thus, an Al-sensitive plant. This, in turn, could decrease biomass productivity. This study deduces that Al may have interfered with nutrient uptake as well as accumulated in the leaves. Since *A. hybridus* is a staple vegetable consumed in the Sub-Saharan region, primary concerns should be to check the transfer of high Al ions to consumers via the food chain. Identification and characterization of specific post-β-Carotene Al-induced stress genes and their gene products as well as comparing these candidate genes/gene products with other Al-stress related genes/gene products deposited in global databases, will be a promising approach to future research in combating the menace of Al toxicity in Sub-Saharan Africa.

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Figures

Figure 1

Growth of Amaranthus hybridus plants in hydroponics

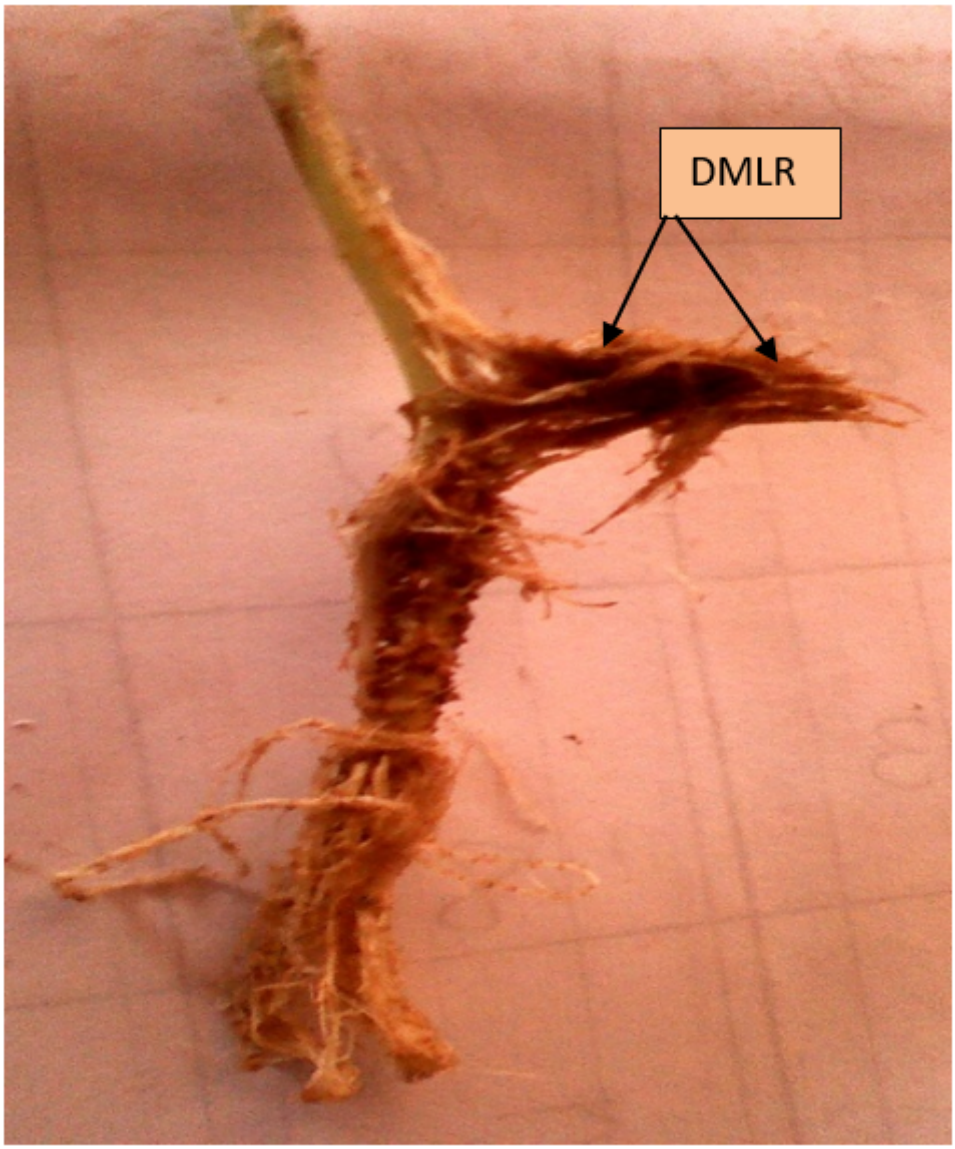

Figure 2

Roots of Al toxicity stressed normal plant enlarged. DMLR=dense mesh of lateral roots (Udengwu and Egedigwe 2014)

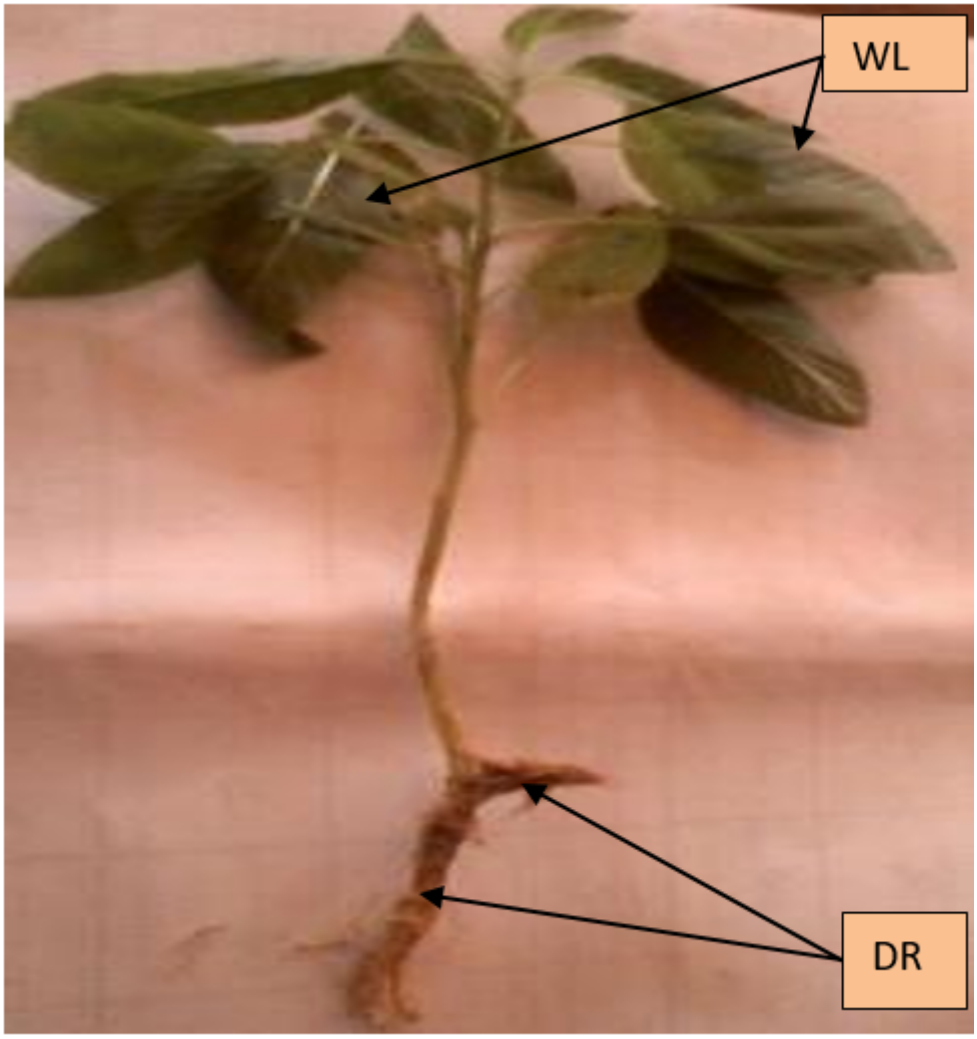

Figure 3

Aluminium toxicity stressed plant WL=Wilting of leaves; DR=Damaged roots (Udengwu and Egedigwe 2014)

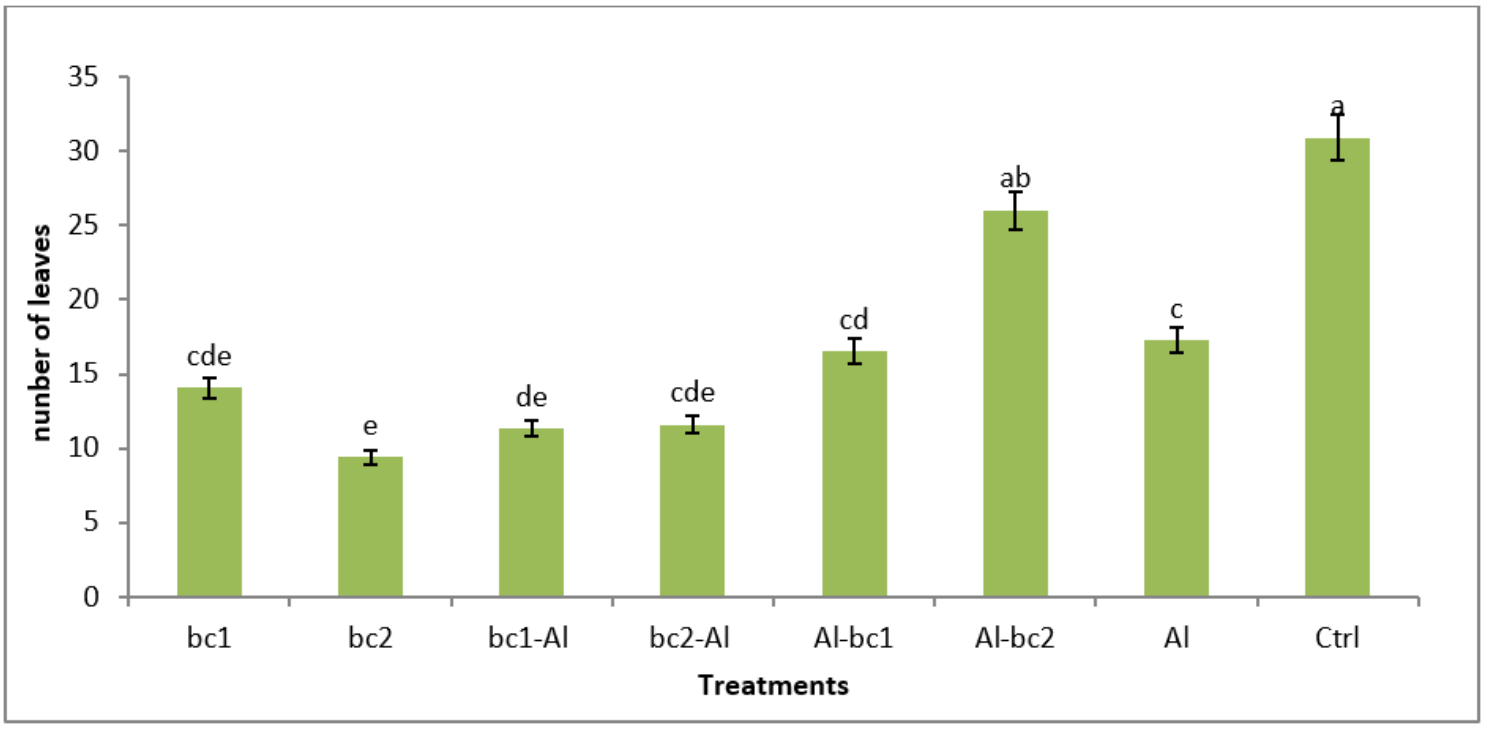

Figure 4

Effects of treatments on the number of leaves (NOL). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

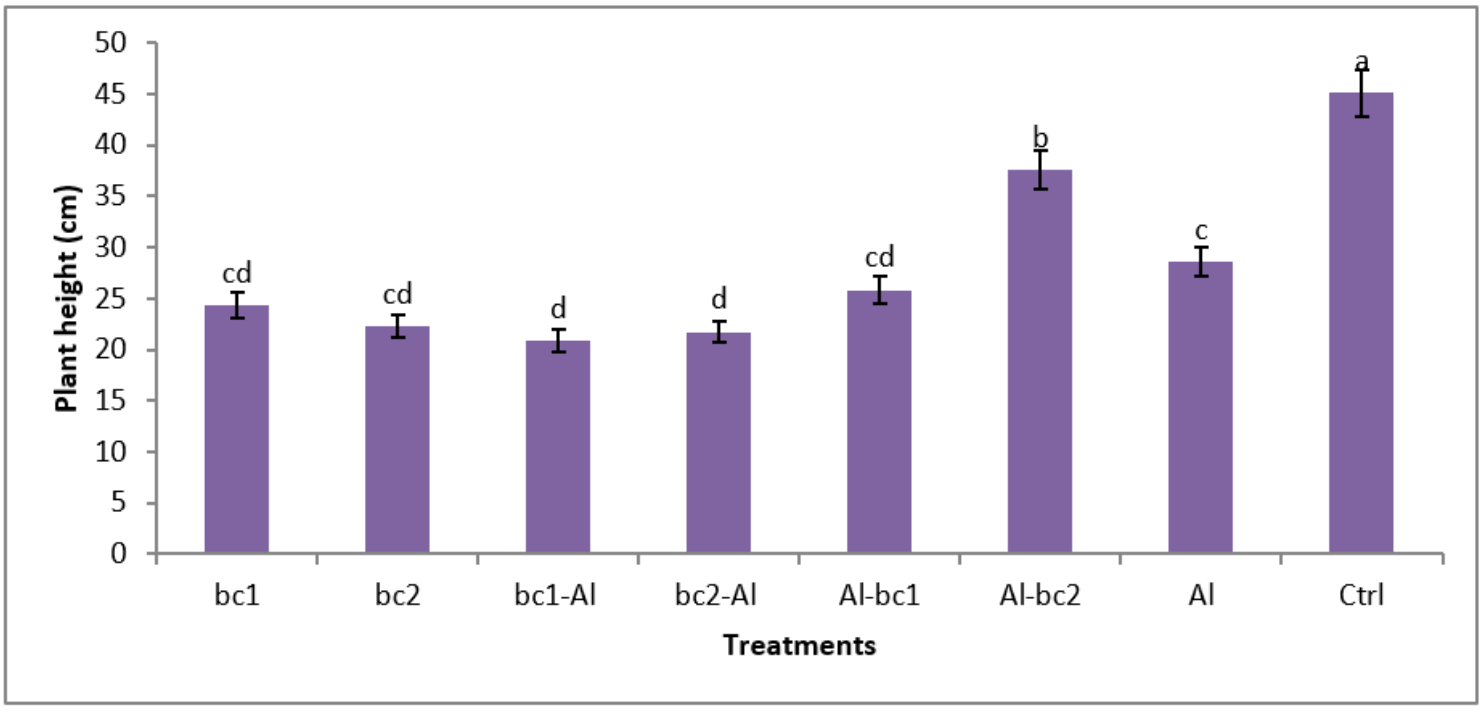

Figure 5

Effects of treatments on plant height (PLH). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

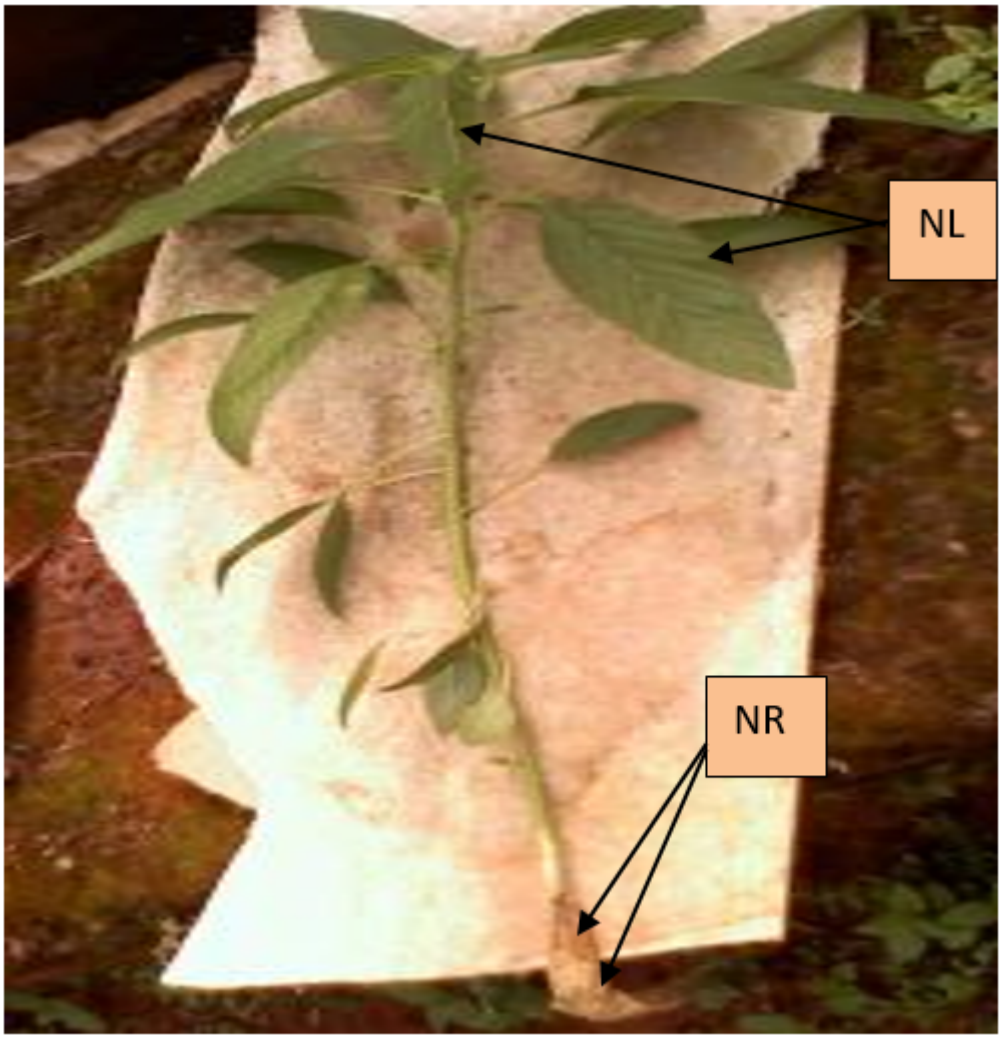

Figure 6

Control Amaranthus plant NL=Normal leaves; NR=normal roots (Udengwu and Egedigwe 2014)

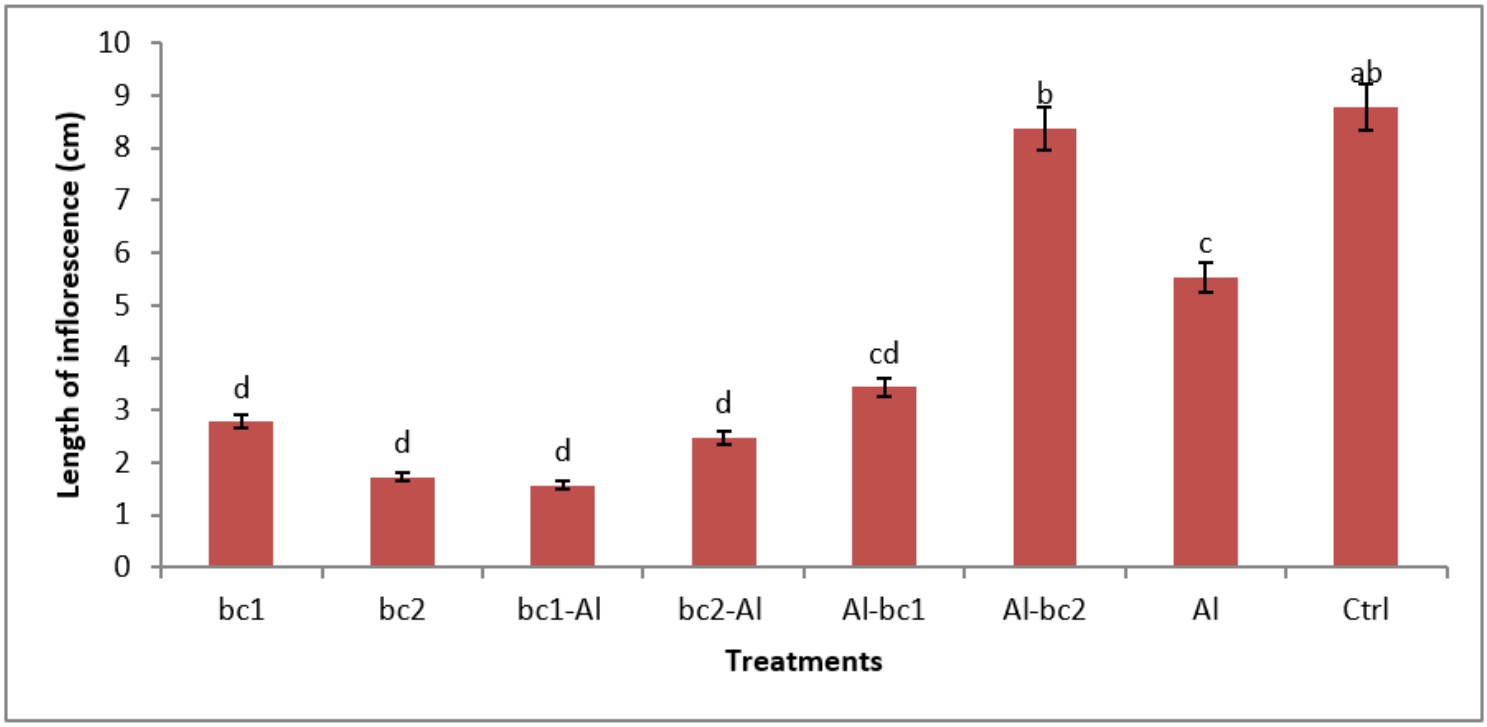

Figure 7

Effects of treatments on the length of inflorescence (LOI). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

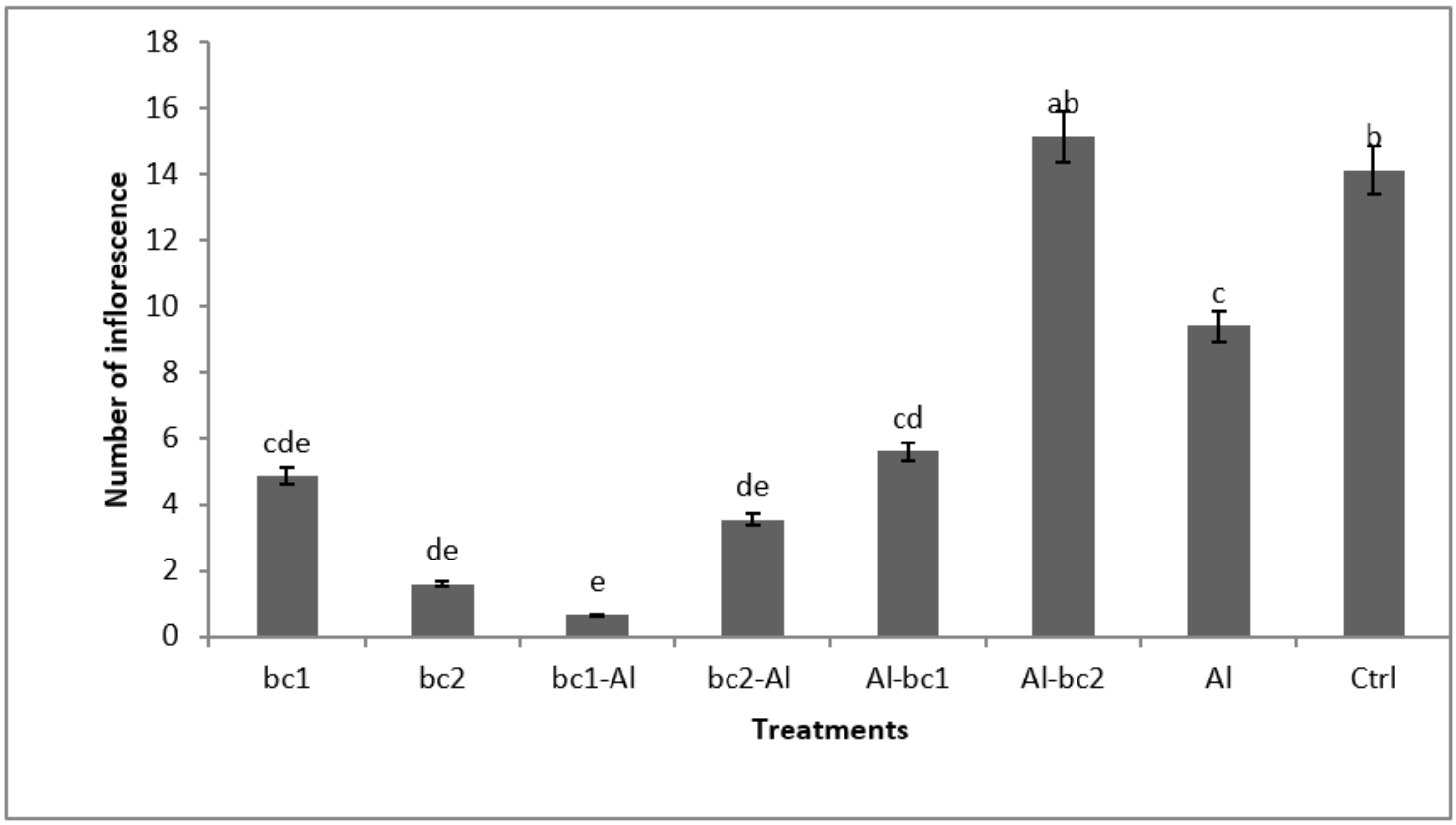

Figure 8

Effects of treatments on the number of inflorescences (NOI). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

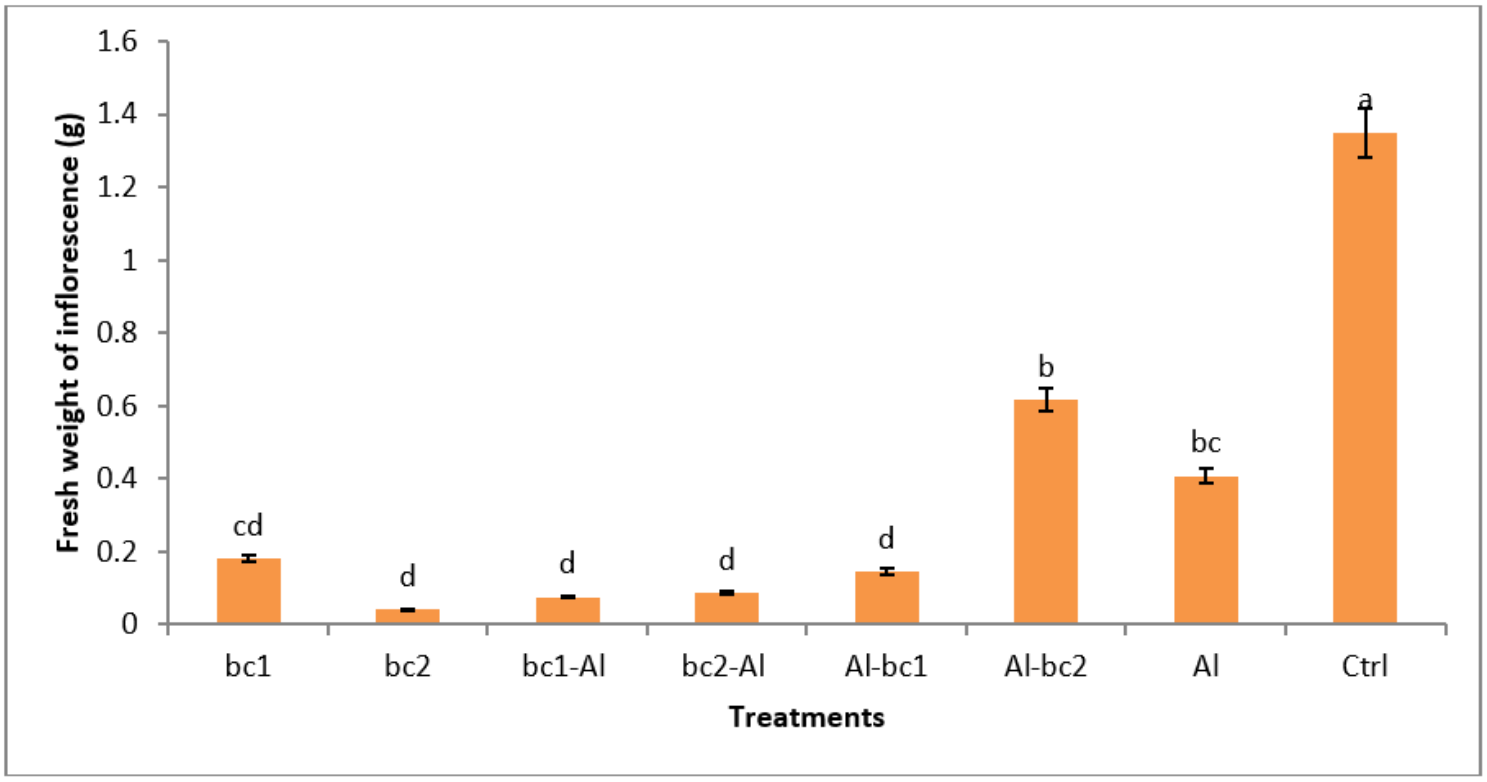

Figure 9

Effects of treatments on fresh weight of inflorescence (FWI). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

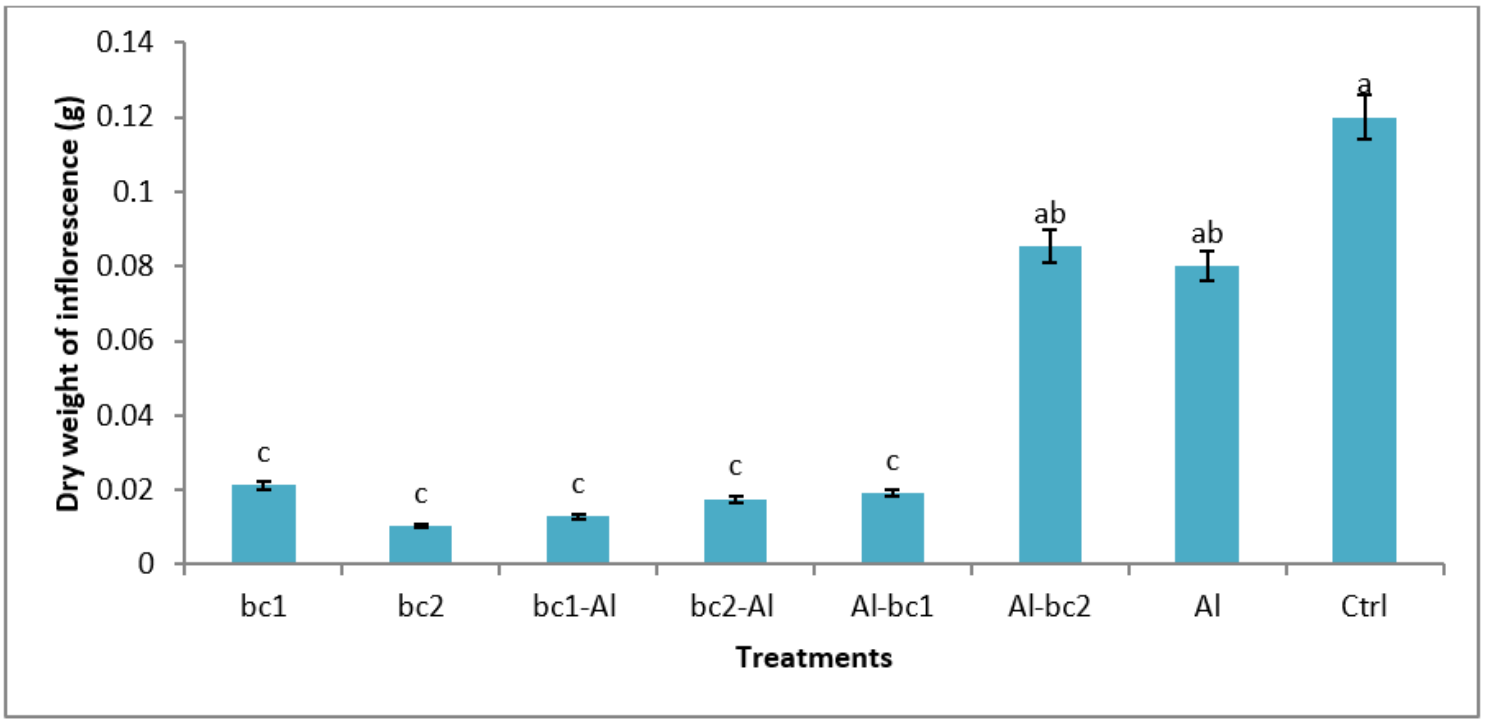

Figure 10

Effects of treatments on the dry weight of inflorescence (DWI). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

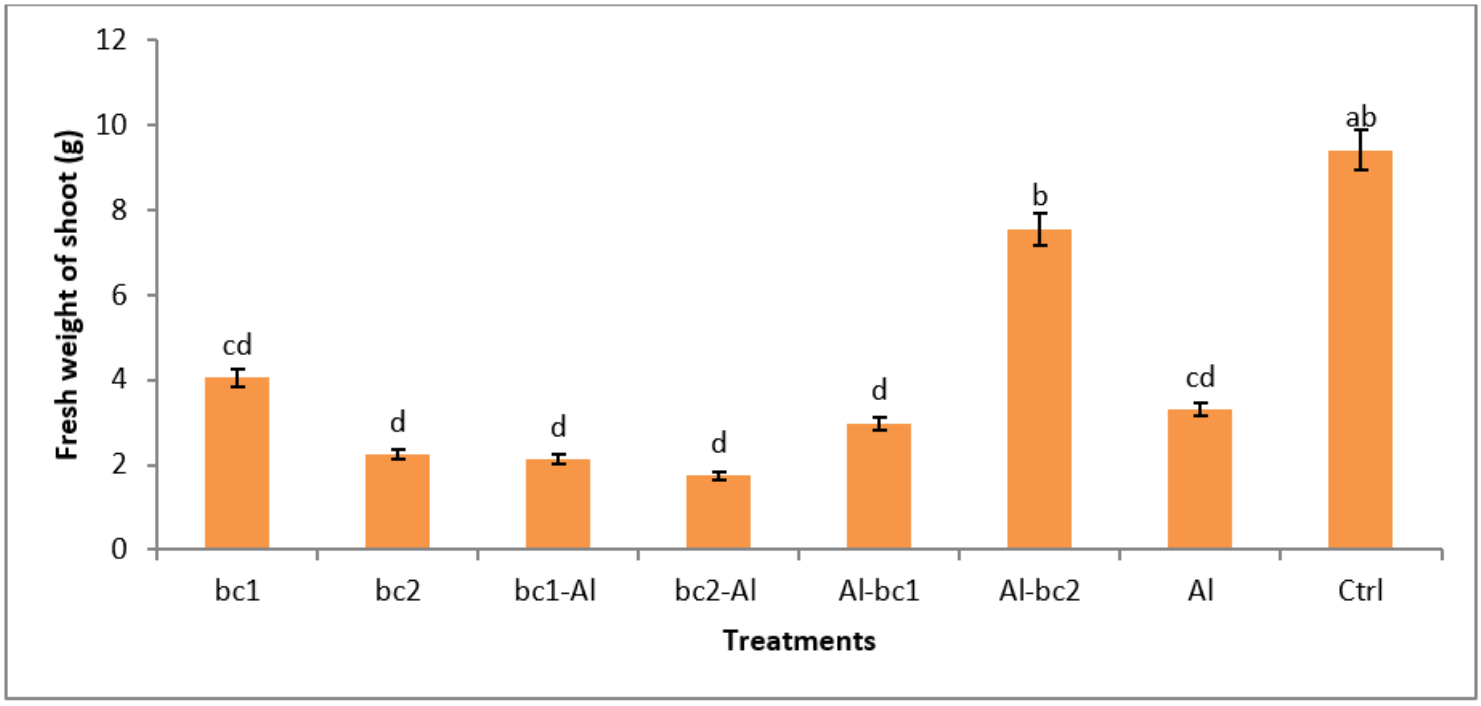

Figure 11

Effects of treatments on fresh weight of shoot (FWI). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

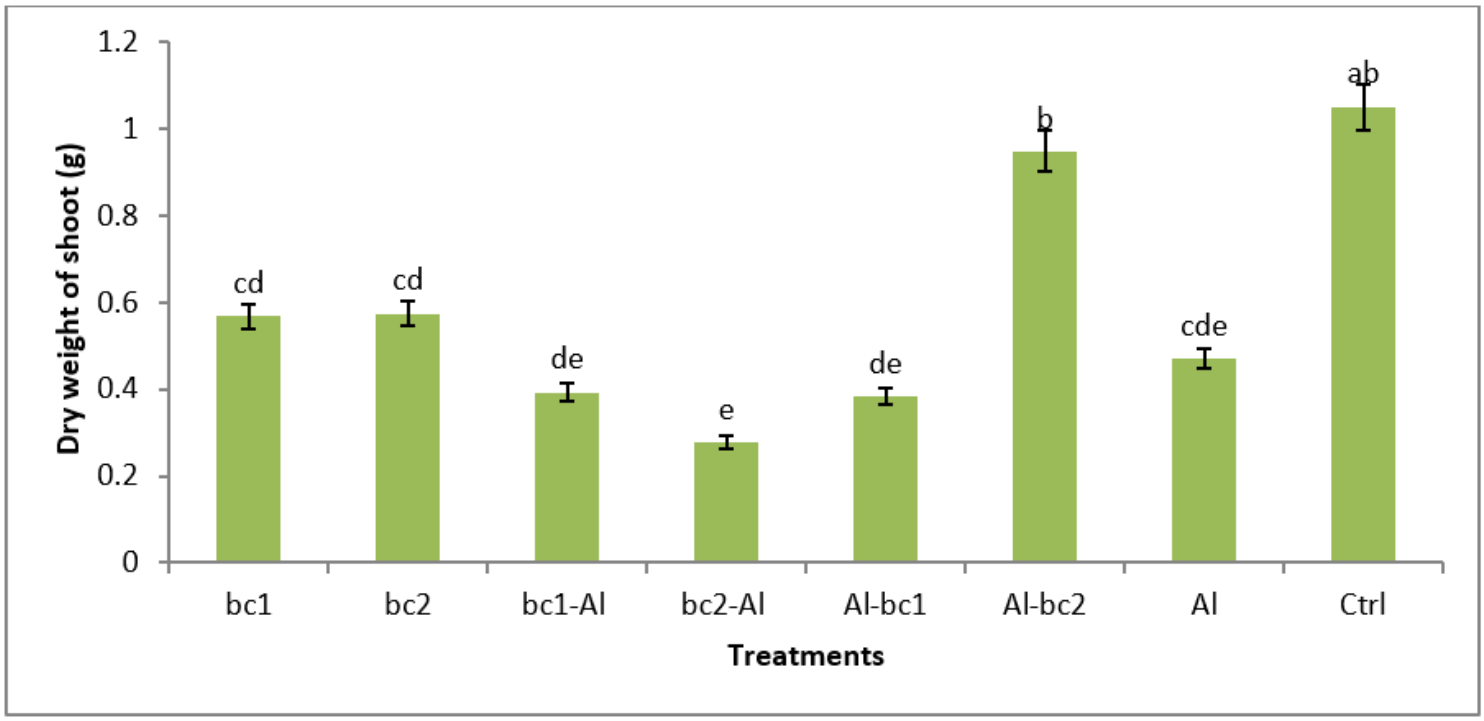

Figure 12

Effects of treatments on the dry weight of shoot (DWS). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

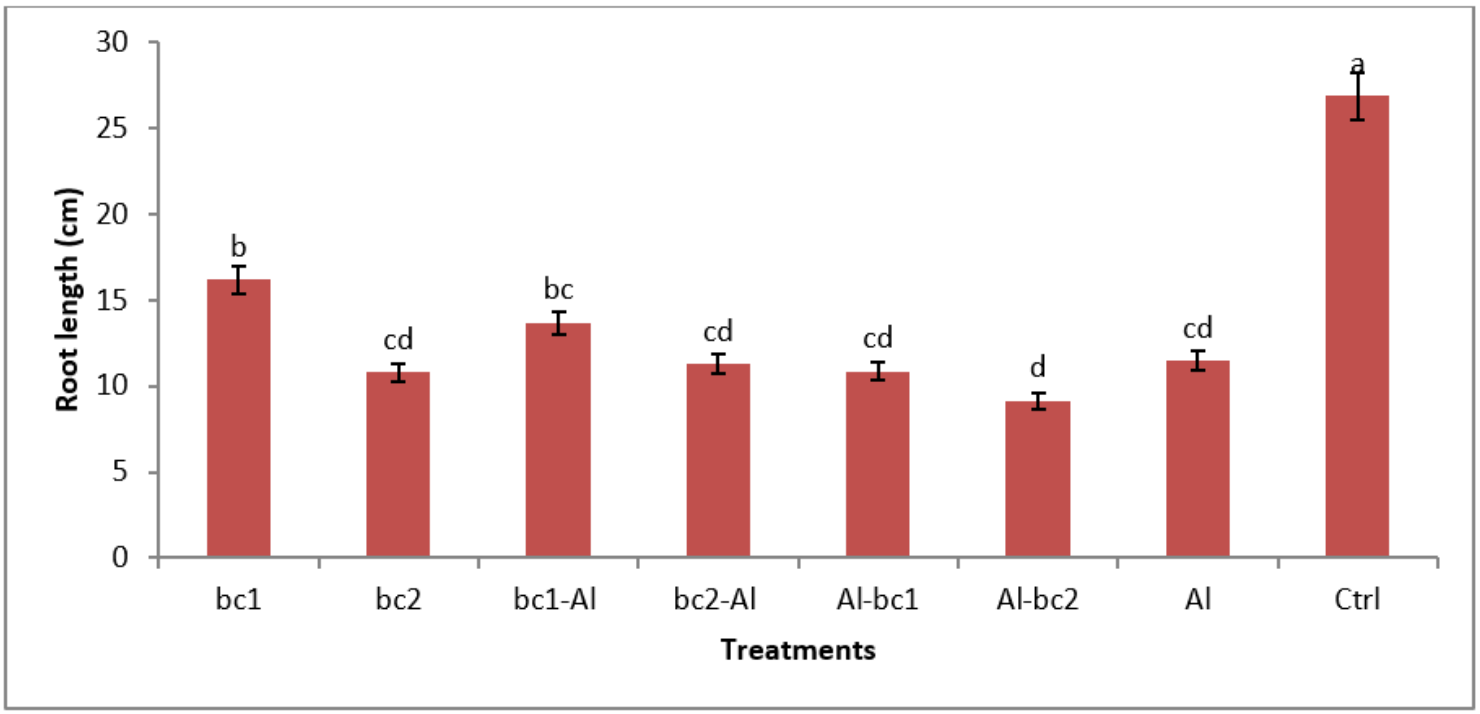

Figure 13

Effects of treatments on root length (ROL). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

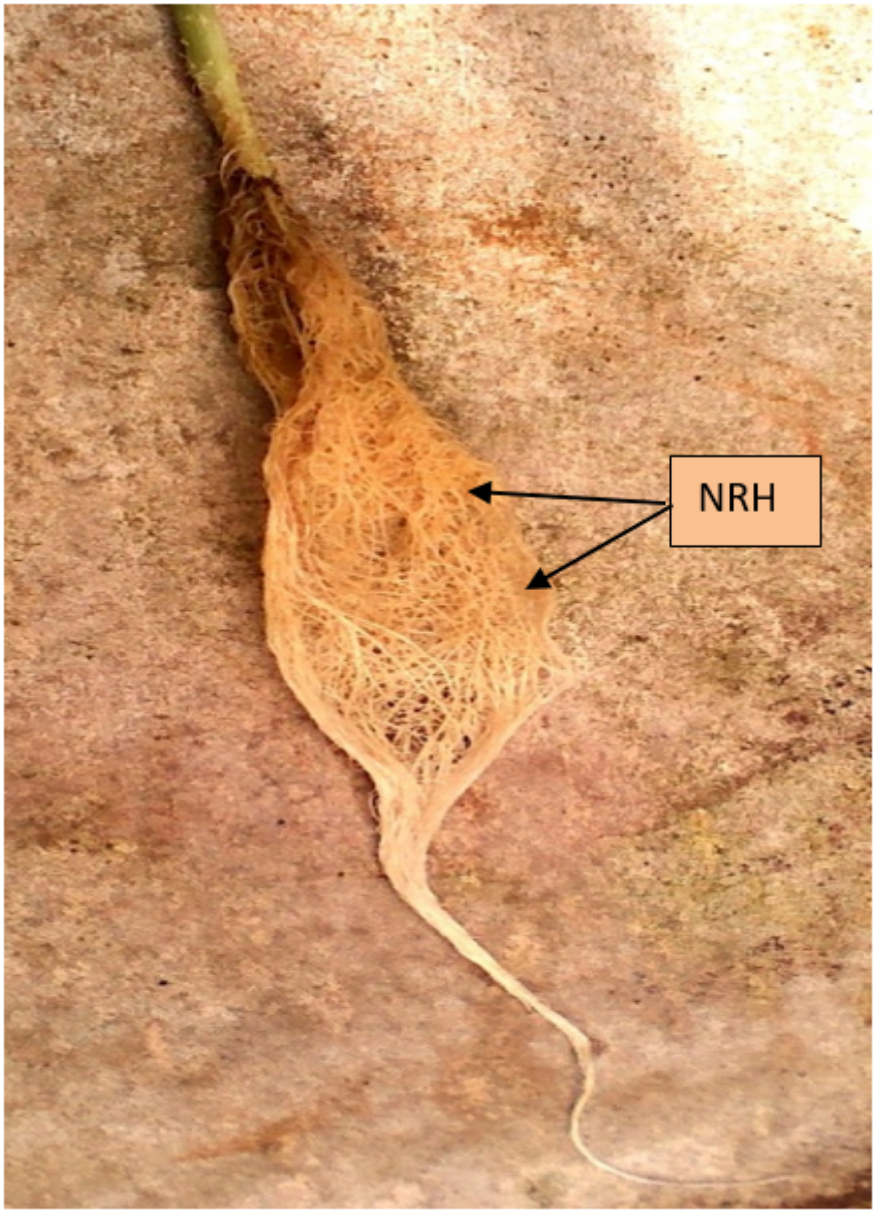

Figure 14

Roots of control plant enlarged. NRH=Normal Roots from Hydroponics (Udengwu and Egedigwe 2014)

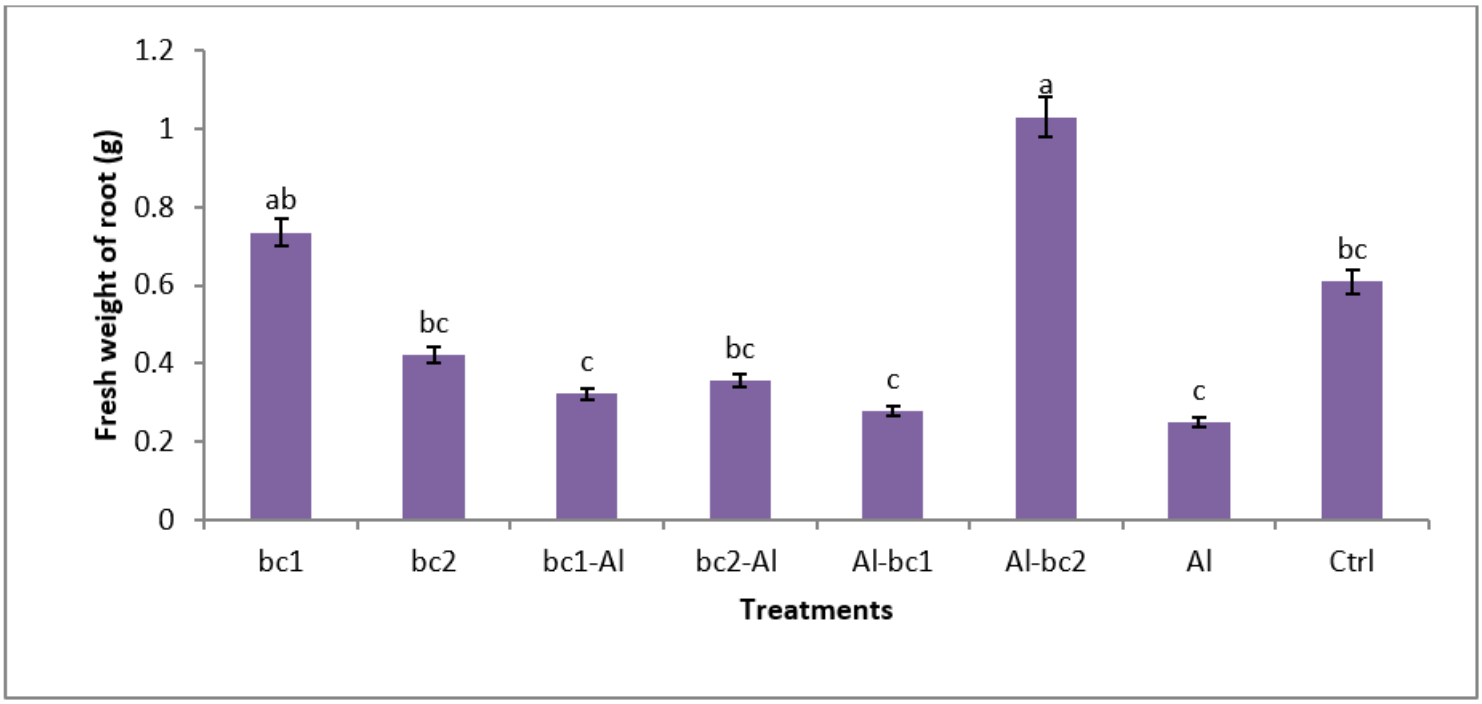

Figure 15

Effects of treatments on fresh weight of root (FWR). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

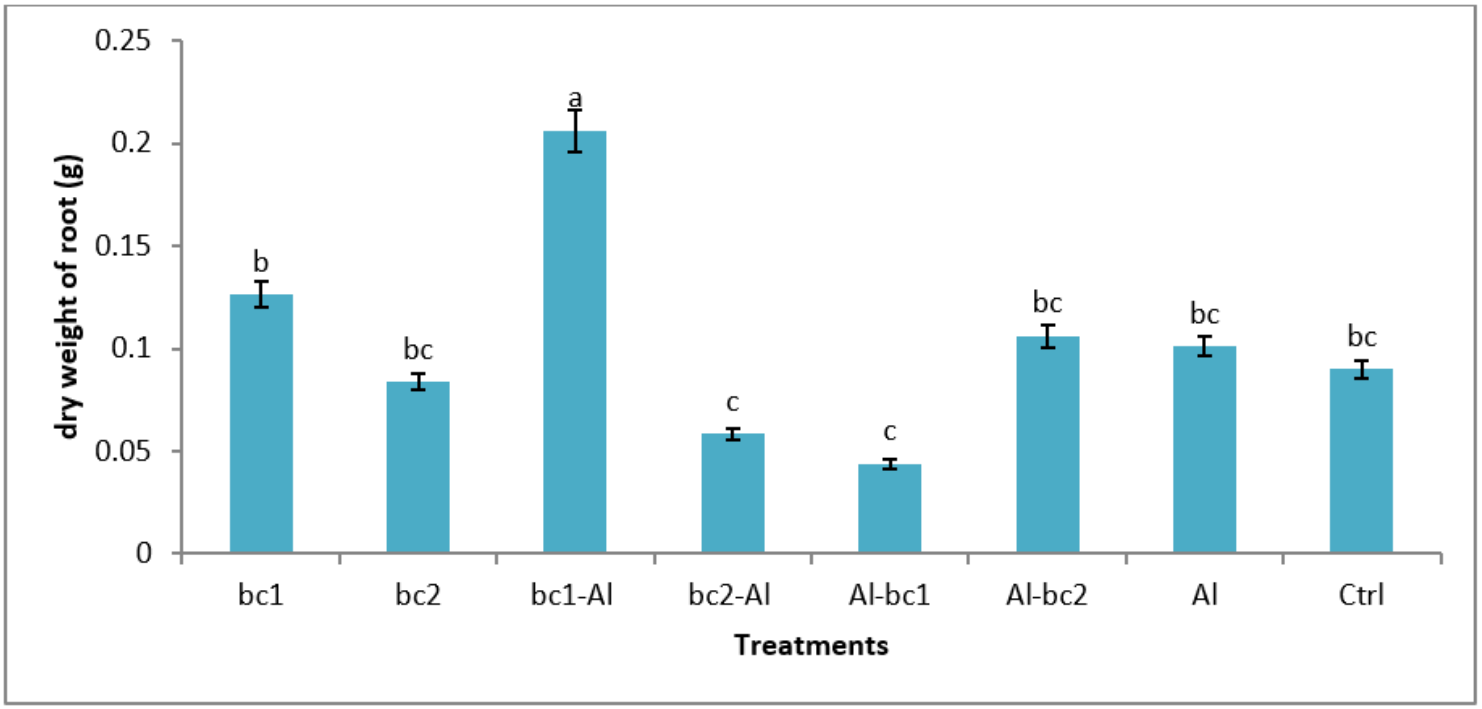

Figure 16

Effects of treatments on the dry weight of root (DWR). *Bars bearing different letters differ significantly (LSD ≤ 0.05)